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SHORT NOTE

Ectomycorrhizal fungi associated with two species of *Kobresia* in an alpine meadow in the eastern Himalaya

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Abstract The diversity of ectomycorrhizal fungi (EMF) on *Kobresia filicina* and *Kobresia capillifolia* in an alpine meadow in China's southwestern mountains, one of the world's hotspots of biodiversity, was estimated based on internal transcribed spacer rDNA sequence analysis of root tips. Seventy EMF operational taxonomical units (OTUs) were found in the two plant species. Dauciform roots with EMF were detected in species of *Kobresia* for the first time. OTU richness of EMF was high in *Tomentella/Thelophora* and *Inocybe*, followed by *Cortinarius*, *Sebacina*, the *Cenococcum geophilum* complex, and *Russula*. *Tomentella/Thelophora* and *Inocybe* were general and dominant mycobiont genera of the two sedges. Besides the *C. geophilum* complex, the ascomycete components *Hymenoscyphus* and *Lachnum* were also detected on the two plants. Alpine plants in different geographical regions share similar main genera and/or families of EMF while harboring predominantly different mycobiont species; most of the members detected by us have not been found elsewhere. Significant differences in the profile of EMF occurrences were not found between the two plant species and among the three sampling seasons in our sample size.

Keywords Mycobiont · *Kobresia* · Alpine meadow · Dauciform root · ITS rDNA

Introduction

Mycorrhizae are likely to be of importance in nutrient-stressed or infertile environments, such as alpine areas. Forming ectomycorrhizal (EM) associations is one of the most ecologically important symbiotic associations in terrestrial ecosystems (Smith and Read 1997; Cairney and Chambers 1999; Rinaldi et al. 2008) and is believed to be a crucial and effective way of alleviating nutritional stress for both plants and fungi in alpine areas. EM are common on alpine woody plants (Wang and Qiu 2006) and have also been detected on several alpine/arctic grasses (Wang and Qiu 2006; Moreau et al. 2006; Li and Guan 2007).

Grasses in the Cyperaceae (the sedge family) are common in stressed habitats like alpine/arctic areas. Recent studies have revealed that many species in the Cyperaceae are mycorrhizal (Muthukumar et al. 2004). Ectomycorrhizal (EM) and/or ectomycorrhizal fungi (EMF) communities on *Kobresia myosuroides* (Villars) Foiri [= *K. bellardii* (All.) Degel] were reported or characterized in many works (Fontana 1963; Haselwandter and Read 1980; Read and Haselwandter 1981; Kohn and Stasovski 1990; Gardes and Dahlberg 1996; Massicotte et al. 1998; Lipson et al. 1999; Schadt and Schmidt 2001; Ali and Hossein 2008; Mühlmann and Peintner 2008b). In addition, many more common sedge species in nutrient-poor environments have been found to form “dauciform roots,” which are specialized structures produced as morphological and physiological adaptations of plants to nutrient adversities and may have a similar function in mycorrhizae in nutrient acquisition (Shane et al. 2006).

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Kobresia filicina (C. B. Clarke) C. B. Clarke and *Kobresia capillifolia* (Decne.) C. B. Clarke, together with other grasses of *Potentilla* and *Polygonum*, are dominant plant species in the easternmost Himalaya and in the mountains of southwest China (Wu and Zhu 1987; Zhou 2001), one of the world's hotspots of biodiversity. Forming mycorrhizal associations may be one of their important means for alleviating nutritional stress in alpine environments. Nothing, however, is known about the EMF status of plants in the region. In addition, it is intriguing to determine whether dauciform roots occur on species of *Kobresia* that grow there and whether they form EM. The objectives of this work were (1) to identify diversity of EMF on the two species of *Kobresia* in an alpine meadow in the region and (2) to determine whether dauciform roots occur on species of *Kobresia* and whether they form EM to survive the alpine stress.

Materials and methods

Sampling and sample processing

The sampling site was an alpine meadow (altitude, 4,300 m) on Hong Shan (27°50'N, 99°24' E), Shangri-La County (Zhongdian) in Yunnan Province, southwest China. Sampling of *K. filicina* and *K. capillifolia* was performed in mid-May (spring), late July (summer), and early September (autumn) 2007. Samples were randomly collected within a 50×50-m² square 5–6 m away from each other. Plants (including their roots and aboveground parts) and surrounding soil were excavated, resulting in plots measuring about 30×20×20 (length, width, and depth) cm each. Ten samples were made for each plant species on each sampling date, resulting in 60 samples in total. The occasionally occurring sporocarps around or near the sampling plots were collected to obtain reference sequences for identification of the EMF.

Ectomycorrhizal root tips were examined at ×3 magnification under a dissecting microscope and macroscopically sorted into morphotypes based on color, mantle surface, ramification pattern, and occurrence of emanating hyphae (Agerer 2006). Dauciform roots were examined and sorted by color. At least 10–20 root tips of an individual morphotype were stored in saturated NaCl/CTAB solution at –20°C until used in molecular investigations.

PCR and sequence analyses of the ITS rDNA region

DNA was extracted from root tips following the procedures of Hibbett and Vilgalys (1993) with several modifications. Primer combinations of ITS1F×ITS4 (Gardes and Bruns 1993), ITS5×ITS4, and ITS1F×LR1 were used to amplify

the rDNA internal transcribed spacer (ITS) region. PCR products were sequenced after cloning.

After blast searching against GenBank and UNITE databases, sequences were sorted into operational taxonomical units (OTUs), which were defined as sequences with at least 97% similarity and regarded as belonging to one species (Mühlmann et al. 2008; Mühlmann and Peintner 2008a, b). Our ITS rDNA sequences are deposited in GenBank as accession numbers FJ581421, FJ581422, and FJ378717–FJ378866.

Statistical analyses

Frequency of an EMF OTU was defined as the number of samples from which the OTU was detected. Chi-square test was performed with SAS software (The SAS system for Windows 9.0), with frequency of OTUs used as the dependent variable, “plant species” and “season” as independent variables, respectively. The significance level was set as 0.05. Diversity measures per sample included diversity index (*H*) of Shannon and Wiener (1949), species richness index (*R*) of Margalef (1958), and evenness index (*E*) of Pielou (1969). Kruskal–Wallis test was performed, with average Shannon and Wiener's diversity index per sample used as the dependent variable, “plant species” and “season” as independent variables, respectively, and the significance level was set as 0.05.

Results

EMF diversity of the two plants

Eight ectomycorrhizal morphotypes were found on the two species of *Kobresia*. Sixty seven EMF OTUs were obtained from their ectomycorrhizal root tips and 11 from the dauciform roots (eight of them were also found on ectomycorrhizal root tips; Table 1). Total OTU richness of EMF (including both plant species and three seasons) was 70. Most (59) were basidiomycetes, and 11 were ascomycetes. OTU richness was high in *Tomentella/Thelophora* (16) and *Inocybe* (15), followed by *Cortinarius* (seven), *Sebacina* (seven), the *Cenococcum geophilum* complex (seven), and *Russula* (five) (Supplementary Figs. 1–4) while low in *Lachnum* (two), *Hymenoscyphus* (two), *Amanita* (two), *Laccaria* (two), *Lactarius* (two), *Hebeloma* (one), and *Boletus* (one). Seventy percent of the total OTUs (49 OTUs) were detected only once, and several OTUs closely matched EMF in Europe (Table 1). It should be noted that the EMF of *Boletus reticuloceps* and *Lactarius* 01 was identified based on its high similarity to the sporocarps of *B. reticuloceps* (FJ548566) and *Lactarius subsphagnetii* (FJ378814), respectively (Table 1).

Table 1 Mycobionts on *K. filicina* and *K. capillifolia*

EMF OTUs	Frequency					Closest match and accession number	
	By plant		By season			In GenBank	In UNITE database
	Kc	Kf	May	July	September		
<i>Amanita</i> 01	1	0	0	1	0	<i>A. velosa</i> (92%) DQ974692	<i>Amanita</i> sp. (1,061 bits) UDB000929
<i>Amanita</i> 02	0	1	0	1	0	<i>A. vaginata</i> (91%) AJ889925	<i>A. mortenii</i> (757 bits) UDB002235
<i>Boletus reticuloceps</i>	0	2	0	2	0	<i>B. reticuloceps</i> (100%) FJ548566	
<i>Sebacina</i> 01	1	0	0	1	0		<i>Sebacina</i> sp. (416 bits) UDB000774
<i>Sebacina</i> 02	1	0	1	0	0	<i>Sebacina</i> EM (83%) AB218165	
<i>Sebacina</i> 03	3	1	3	0	1	<i>Sebacina</i> EM (97%) AF440652	<i>Sebacina</i> sp. (660 bits) UDB000774
<i>Sebacina</i> 04	0	1	1	0	0	<i>Sebacina</i> EM (98%) AF440648	<i>Sebacina</i> sp. (789 bits) UDB000773
<i>Sebacina</i> 05	1	1	0	0	2	<i>Sebacina</i> EM (97%) AF440648	<i>Sebacina</i> sp. (765 bits) UDB000773
<i>Sebacina</i> 06	0	1	0	0	1	<i>Sebacina</i> sp. (95%) EF433975	
<i>Sebacina</i> 07	1	0	0	1	0	<i>Sebacina</i> EM (96%) AY940653	
<i>Cortinarius</i> 01	1	0	0	0	1	<i>C. psammocephalus</i> (95%) AY669672	
<i>Cortinarius</i> 02	0	1	0	1	0	<i>Cortinarius</i> EM (98%) AY641471	<i>C. psammocephalus</i> (1,118 bits) UDB002161; <i>C. diasemospermus</i> (1,172 bits) UDB001230
<i>Cortinarius</i> 03	1	1	0	0	2	<i>C. umbrinolens</i> (95%) AY669658; <i>Cortinarius</i> EM (99%) AY748857	
<i>Cortinarius</i> 04	0	1	1	0	0	<i>C. flexipes</i> (96%) AJ889971	<i>C. flexipes</i> (1,029 bits) UDB000063
<i>Cortinarius</i> 05	1	0	0	0	1	<i>C. cf. saniosus</i> (98%) DQ102683	<i>C. rubroviolipes</i> (1,074 bits) UDB001453
<i>Cortinarius</i> 06	1	0	0	0	1	<i>C. cf. saniosus</i> (98%) DQ102683	<i>C. diasemospermus</i> (1,150 bits) UDB001230
<i>Cortinarius</i> 07	0	1	0	0	1	<i>C. parvannulatus</i> (89%) AY669664	<i>C. diasemospermus</i> (731 bits) UDB001230
<i>Inocybe</i> 01	1	0	0	0	1	<i>I. pudica</i> (89%) AY228341	
<i>Inocybe</i> 02	0	2	0	2	0	<i>Inocybe</i> EM (88%) EF218781	
<i>Inocybe</i> 03	2	1	1	2	0	<i>Inocybe</i> EM (94%) EF641838	<i>I. rimosa</i> (1,017 bits) UDB000103
<i>Inocybe</i> 04	1	0	1	0	0	<i>Inocybe</i> EM (94%) EF641838	<i>I. rimosa</i> (987bits) UDB000103
<i>Inocybe</i> 05	1	1	1	0	1	<i>Inocybe</i> EM (93%) AY940653; <i>I. egenula</i> (95%) AM882714	
<i>Inocybe</i> 06	2	2	3	0	1	<i>Inocybe</i> EM (91%) EF218773	<i>I. aurea</i> (521 bits) UDB000612
<i>Inocybe</i> 07 (<i>I. umbrina</i>)	0	1	1	0	0	EM (89%) AB218065	<i>I. umbrina</i> (545 bits) UDB000608
<i>Inocybe</i> 08 (<i>I. acutella</i>)	1	0	0	0	1	<i>I. acutella</i> (96%) AM882923	<i>I. acutella</i> (910 bits) UDB000609
<i>Inocybe</i> 09	1	1	1	1	0	<i>I. cf. lanuginosa</i> (92%) EU525979	
<i>Inocybe</i> 10	0	1	0	0	1	<i>I. cf. hirculus</i> (92%) AM882986	
<i>Inocybe</i> 11	1	0	1	0	0	<i>I. egenula</i> (96%) AM882714	
<i>Inocybe</i> 12	1	2	2	1	0	<i>I. egenula</i> (95%) AM882714; <i>Inocybe</i> EM (95%) AY940653	
<i>Inocybe</i> 13	1	0	1	0	0	<i>I. egenula</i> (95%) AM882714	
<i>Inocybe</i> 14	0	2	1	0	1	<i>I. pudica</i> (89%) AY228341; EM(93%) AY940653	
<i>Inocybe</i> 15	0	1	0	1	0	<i>I. aff. lanuginosa</i> (93%) EU486457	<i>I. calamistrata</i> (777 bits) UDB001195
<i>Hebeloma</i> 01	1	2	0	0	3	<i>H. mesophaeum</i> (97%) AB211272; <i>H. albocolossum</i> (99%) AY308583	<i>H. velutipes</i> (918 bits) UDB002445
<i>Laccaria</i> 01	1	0	0	1	0	<i>L. bicolor</i> (98%) DQ097876	<i>L. amethystine</i> (1,394 bits) UDB000006
<i>Laccaria</i> 02	1	0	0	0	1	EM (94%) AB218097	<i>L. laccata</i> (1,255 bits) UDB000106
<i>Tomentella</i> 01	1	0	0	0	1	<i>Tomentella</i> EM (95%) EF218826	<i>T. bryophila</i> (955 bits) UDB000035
<i>Tomentella</i> 02	1	0	0	1	0	<i>Tomentella</i> EM (93%) EF218826	<i>Tomentella</i> sp. (904 bits) UDB001658
<i>Tomentella</i> 03	2	1	3	0	0	<i>T. ramosissima</i> (95%) U83480	<i>T. lapida</i> (1,088 bits) UDB001657
<i>Tomentella</i> 04	1	0	1	0	0	<i>T. ramosissima</i> (93%) U83480	<i>T. lapida</i> (920 bits) UDB001657
<i>Tomentella</i> 05	1	0	0	1	0	Thelephoraceae EM (93%) AY825525	<i>T. caryophyllea</i> (886 bits) UDB000119
<i>Tomentella</i> 06	0	1	1	0	0	<i>Thelephora</i> EM (96%) EF655695	<i>T. caryophyllea</i> (932 bits) UDB000119
<i>Tomentella</i> 07	1	0	1	0	0	Thelephoraceae EM (92%) EF825525	<i>T. penicillata</i> (1,080 bits) UDB000775

Table 1 (continued)

EMF OTUs	Frequency					Closest match and accession number	
	By plant		By season			In GenBank	In UNITE database
	Kc	Kf	May	July	September		
<i>Tomentella</i> 08	1	0	0	1	0	<i>T. caryophyllea</i> (94%) AJ889980	<i>T. caryophyllea</i> (1,049 bits) UDB000119
<i>Tomentella</i> 09	2	1	1	1	1	<i>Tomentella</i> EM (96%) EF218831	<i>T. bryophila</i> (831 bits) UDB000035
<i>Tomentella</i> 10	3	0	0	0	3	Thelephoraceae EM (97%) EF077519	<i>T. stiposa</i> (1,065 bits) UDB000248
<i>Tomentella</i> 11	1	0	0	0	1	<i>Tomentella</i> EM (100%) EF218830	<i>T. badia</i> (914 bits) UDB001656
<i>Tomentella</i> 12	1	0	1	0	0	<i>Tomentella</i> EM (100%) EF218830	<i>T. atramentaria</i> (1,088 bits) UDB000235
<i>Tomentella</i> 13 (<i>T. atramentaria</i>)	1	0	1	0	0	<i>Tomentella</i> EM (98%) AY748876	<i>T. atramentaria</i> (1,013 bits) UDB000955
<i>Tomentella</i> 14 (<i>T. badia</i>)	0	1	1	0	0	<i>Tomentella</i> EM (100%) EF218830	<i>T. badia</i> (1,037 bits) UDB001656
Thelephoraceae 01	1	0	1	0	0	<i>Tomentella</i> EM (100%) EF218830	<i>T. badia</i> (599 bits) UDB000961
Thelephoraceae 02	0	1	0	1	0	<i>Tomentella</i> EM (86%) EU326163	
<i>Russula</i> 01	1	1	1	1	0	<i>R. cuprea</i> (97%) AY061667	<i>R. cuprea</i> (1,170 bits) UDB002457
<i>Russula</i> 02	0	1	1	0	0		<i>R. sanguinea</i> (965 bits) UDB001634
<i>Russula</i> 03	5	2	1	0	6	Russulaceae sp. (97%) DQ061886	<i>R. chloroides</i> (1,207 bits) UDB002496
<i>Russula</i> 04	2	0	0	0	2	<i>Russula</i> EM (97%) EF218798	<i>R. chloroides</i> (1,164 bits) UDB002496
<i>Russula</i> 05 (<i>R. pallescens</i>)	1	0	1	0	0	<i>R. pallescens</i> (96%) DQ421987	<i>R. pallescens</i> (1,047 bits) UDB002461
<i>Lactarius</i> 01 (<i>L. subsphagneti</i>)	1	3	0	3	1	<i>L. subsphagneti</i> (99%) FJ378814	<i>L. aurantiacus</i> (1,411 bits) UDB000887
<i>Lactarius</i> 02 (<i>L. spinosulus</i>)	0	1	0	0	1	<i>L. spinosulus</i> (97%) AY606955	<i>L. spinosulus</i> (1,366 bits) UDB000373
Russulaceae 01	1	0	0	0	1	<i>L. spinosulus</i> (96%) AY606955	<i>L. spinosulus</i> (807 bits) UDB000373
<i>Hymenoscyphus</i> 01	0	1	0	0	1	Ascomycete EM (98%) AJ534703	<i>H. ericae</i> (886 bits) UDB000515
<i>Hymenoscyphus</i> 02	1	2	1	1	1		<i>H. ericae</i> (694 bits) UDB000509
<i>Lachnum</i> 01	2	1	3	0	0		<i>L. brevipilosum</i> (1,011 bits) UDB003074
<i>Lachnum</i> 02	1	1	0	0	2		<i>L. brevipilosum</i> (1,041 bits) UDB003074
<i>C. geophilum</i> 01	1	0	0	1	0	<i>C. geophilum</i> (92%) AY394919	<i>C. geophilum</i> (654 bits) UDB002301
Hymenogastraceae 01 ^a	1	3	2	0	2	<i>Hymenogaster rubyensis</i> (91%) AY945303	
Helotiales 01 ^a	0	2	1	0	1	Helotiales sp. (97%) EF093147	
Helotiales 02 ^a	0	1	1	0	0	Helotiales EM (99%) EU326174	
Helotiales 03 ^a	0	1	1	0	0	Helotiales sp. (97%) EF093147	
<i>Acephala</i> sp. ^a	0	1	0	0	1	<i>Acephala</i> sp. (98%) EU434831	<i>Hysterona viascirpina</i> (904 bits) UDB003025
<i>Phialocephala</i> sp. ^a	0	1	1	0	0	<i>P. sphaeroides</i> (90%) EU434851	
<i>Leptodontidium</i> sp. ^a	0	1	0	0	1	<i>L. orchidicola</i> (98%) EU436691	
<i>Pleiochaeta</i> sp. ^a	1	0	0	0	1	<i>P. ghindensis</i> (97%) EU167561	
<i>Cistella</i> sp. ^a	0	1	0	0	1		<i>C. fugiens</i> (654 bits) UDB003082
<i>Naeviopsis</i> sp. ^a	2	5	3	1	3	Helotiales sp. (98%) EF093150	<i>N. arctica</i> (900 bits) UDB003042
<i>Microglossum</i> sp. ^a	2	0	1	0	1	<i>M. viride</i> (99%) AY144534	<i>Psilocistella alchemillae</i> (559 bits) UDB003089
<i>Hyalacrotis</i> sp. ^a	1	0	0	0	1		<i>H. hamulata</i> (892 bits) UDB003006
<i>Pseudeurotium</i> sp. ^a	0	1	0	0	1	<i>Pseudeurotium backeri</i> (99%) DQ068995	

Identification, frequency of OTUs, best blast matches in GenBank, and/or UNITE with identity (percent or bits number) and accession number are shown. Frequency by plant species included samples from all three seasons; frequency by season included samples of both plant species. OTUs in bold include individuals isolated from dauciform roots. May, July, and September are the sampling dates

Kf, *K. filicina*; Kc, *K. capillifolia*

^a Probably plant endophytes

Table 2 Average EMF diversity measures per sample on *K. filicina* and *K. capillifolia*

Index	Samples/species		Samples/season/species					
	Kf	Kc	Kf			Kc		
			May	July	Sept.	May	July	Sept.
Richness	1.253	1.288	1.363	1.165	1.204	1.252	1.430	1.252
Diversity	0.667	0.672	0.679	0.659	0.663	0.668	0.686	0.667
Evenness	0.964	0.968	0.980	0.950	0.955	0.964	1	0.964

Diversity measures including species richness index, diversity index and evenness index are presented for samples of each plant species, and for samples of each season for each plant species separately

Kf, *K. filicina*; Kc, *K. capillifolia*

Thirty-nine EMF OTUs assigned to 12 genera were found on *K. filicina* and 54 of 12 genera were found on *K. capillifolia*. OTU richness was high in *Inocybe* (ten) for *K. filicina* and high in *Tomentella/Thelophora* (13) and *Inocybe* (12) for *K. capillifolia*. Twenty OTUs occurred on both plant species; richness was high in *Inocybe* (six).

For *K. filicina*, 16 OTUs assigned to seven genera were detected from samples collected in May, 12 of nine genera from July, and 15 of nine genera from September. OTU richness was high in *Inocybe* (five) and *Tomentella/Thelophora* (three) for May, high in *Inocybe* (three) for July, and high in *Inocybe* (four) for September. Four OTUs were present during more than one season.

For *K. capillifolia*, 24 OTUs assigned to seven genera were detected from samples of May, 13 of seven genera from July, and 23 of ten genera from September. OTU richness was high in *Tomentella/Thelophora* (seven) and *Inocybe* (six) for May, high in *Inocybe* (four) and *Tomentella/Thelophora* (three) for July, and high in *Cortinari* (four), *Inocybe* (three), and *Tomentella/Thelophora* (four) for September. Six OTUs were present during more than one season.

According to the chi-square test, there were no statistically significant differences of EMF occurrence between the two plant species ($p=0.4211$ for samples collected in all seasons; $p=0.8079$ for samples in May, $p=0.7064$ for July,

and $p=0.2799$ for September) and among the three sampling seasons ($p=0.7640$ for *K. filicina*, $p=0.2856$ for *K. capillifolia*). According to the Kruskal–Wallis test, there were no statistically significant differences of average Shannon and Wiener's diversity indices per sample between the two plant species ($p=0.6106$ for samples of all seasons; $p=0.3042$ for samples in May, $p=0.0555$ for July, and $p=0.6862$ for September) and among seasons ($p=0.2902$ for *K. filicina* and $p=0.2676$ for *K. capillifolia*). Average diversity measures per sample by plant species and by season are shown in Table 2.

Dauciform roots with EMF

Dauciform roots were detected in 19 samples (31.7% of the total samples) of the two species of *Kobresia*. They were carrot-shaped lateral roots generally connected to the parent roots by a peduncle and white, pale yellow, orange, or beige (Fig. 1a). Some of the dauciform roots extending directly from the parent roots without a peduncle were white or occasionally beige. They were initially smooth but turned brush-like due to long, dense root hairs when mature (Fig. 1b). Typical ectomycorrhizal characters, i.e., mantle and Hartig's net, were not detected on them, but fungal hyphae were observed on their surface (Fig. 1c). Eleven EMF OTUs were obtained from them, including members

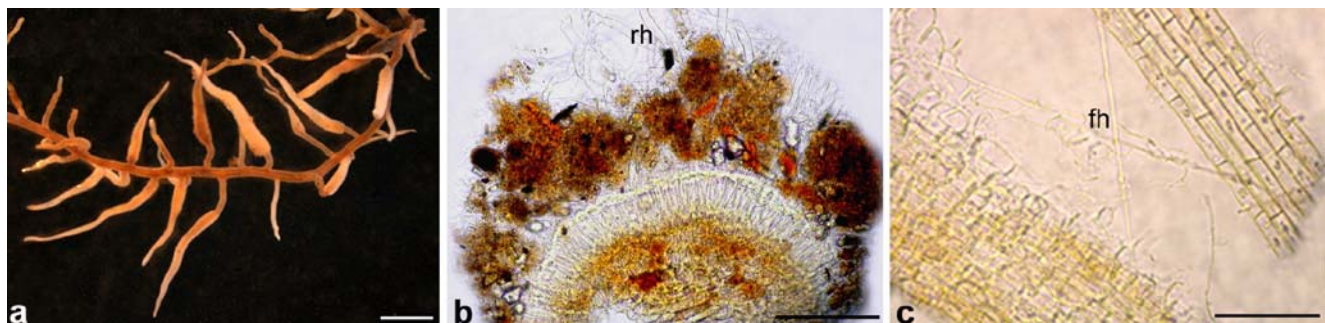


Fig. 1 Dauciform roots of *K. filicina* with EMF. **a** Macromorphology of dauciform roots. **b** Transverse section of a dauciform root (rh root hairs). **c** Vertical section of a dauciform root (fh fungal hyphae). Scale bar is 1 mm for **a**, 250 μm for **b**, and 500 μm for **c**

in *Russula*, *Lactarius*, *Tomentella/Thelophora*, *Cortinarius*, *Sebacina*, *Hymenoscyphus*, and *Lachnum*. OTU richness was high in Russulaceae (four).

Discussion

Mycobiont diversity of alpine plants

Based on former studies of alpine areas in Europe and North America (Gardes and Dahlberg 1996; Schadt and Schmidt 2001; Mühlmann et al. 2008; Mühlmann and Peintner 2008a, b), *Inocybe*, *Cortinarius*, *Tomentella/Thelophora*, *Russula*, and *Lactarius* are the main arctic-alpine ectomycorrhizal genera. Our study in an alpine meadow of southwest China found a similar assemblage. It is evident that alpine plants in different geographical regions share similar main mycobiont genera and/or families. There were regional differences at the species level, however, since most of the EMF OTUs detected have not been found elsewhere.

In our study, the ascomycete mycobionts *Hymenoscyphus* and *Lachnum* were detected on the two species of *Kobresia*, suggesting that further attention to ascomycete mycobionts in addition to the *C. geophilum* complex is needed in future studies. Other ascomycetes, including species of Helotiales (besides *Hymenoscyphus* spp. and *Lachnum* spp.), were detected on ectomycorrhizal root tips and the dauciform roots of the two species of *Kobresia*, and ascomycete mycobionts of Helotiales, Leotiales, Erysiphales, Pezizales (e.g., *Helvella* sp. and *Terfezia boudieri*), and *Lecythophora* were found on several alpine plants (i.e., *K. myosuroides*, *Polygonum viviparum*, and *Salix herbacea*) (Schadt and Schmidt 2001; Ali and Hossein 2008; Mühlmann et al. 2008; Mühlmann and Peintner 2008a, b). Those ascomycetes may be plant endophytes (dark septate fungi and/or arbuscular mycorrhizae), indicating that fungi of different functions, i.e., multiple infections, may coexist within mycorrhizal root tips (Menkis et al. 2005; Wagg et al. 2008) or, alternatively, are opportunistic infections.

General mycobionts of alpine plants

No specificity by EMF communities for plant species and sampling season was detected in our sample size. Similar OTU richness of *Inocybe* was found on the two plant species during the three sampling seasons, and *Tomentella/Thelophora* and *Inocybe* were OTU-rich genera for each species and for each season/each plant. Mühlmann et al. (2008) and Mühlmann and Peintner (2008a, b) also found that *Tomentella/Thelophora* and *Inocybe* were species-rich mycobiont genera of alpine plants in Europe. Thus, the two may be the general and dominant mycobiont genera of

plant species during all seasons plus being generalists with more ecological plasticity to environmental changes than other microbionts in alpine areas.

Dauciform roots colonized by EMF

Our observations revealed that dauciform roots were produced by two species of *Kobresia*, and it is noteworthy that they were colonized by EMF. Dauciform roots, commonly produced in nutrient-impovertished soils, are able to enhance nutrient acquisition of plants. They have been observed on several other plants in the Cyperaceae (e.g., *Caustis blakei* and *Schoenus unispiculatus*) in response to phosphorus deficiency (Playsted et al. 2006; Shane et al. 2004). Dauciform roots colonized by EMF, however, have not been reported previously. For both plants and fungi, production of dauciform roots with EMF might be the result of ecophysiological adaptation to alpine adversity, where environmental conditions cause difficulties in nutrient acquisition.

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